The dargyrome of the genus *Euplotes* (Hypotrichida, Ciliophora)

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Introduction

Since the advent of silver impregnation (Chatton & Lwoff, 1930) to reveal the infraciliature of ciliated protozoa, several previously undetected cortical characteristics have been postulated to be of taxonomic value. Tuffrau (1960) was the first worker to apply silver-staining techniques to hypotrich ciliates of the genus *Euplotes* Ehrenberg, 1831,* concentrating mainly on the prominent ventral ciliature. However, he also revealed the pattern of the fibrillar network of silver-lines that covers both the ventral and dorsal surfaces, introducing the term argyrome to describe the totality of this feature, now known to mark the boundaries between adjacent subpellicular vacuoles (Ruffolo, 1976*a*, 1976*b*). Tuffrau classified the dorsal argyrome (hereafter, dargyrome) patterns into three distinct types, and he applied this classification towards the resolution of taxonomic problems involving several large, common and popular freshwater species. In particular, Tuffrau (1960) suggested that *E. patella* (Müller, 1773) Ehrenberg, 1838 could be distinguished from *E. eurystomus* (Wrzesniowski, 1870) Kahl, 1932 by distinct differences in their dargyrome patterns.

On the basis of a retrospective study of the genus, incorporating many taxonomic descriptions that had accumulated since the introduction of silver-impregnation techniques, Curds (1975) presented a refinement of Tuffrau's (1960) dargyrome classification, raising the number of types to six (Fig. 1). Tuffrau's original classification was based essentially on the number of rows of silver-staining polygons (corresponding to subpellicular vacuoles) that occur between any two adjacent kineties (rows of cilia). In Tuffrau's 'vannus' dargyrome type, there is only a single row (as in *E. vannus* (Müller, 1786) Minkjewicz, 1901), while in his 'eurystomus' type, there are two rows, separated by an interkinetal vacuolar boundary. Curds (1975) retained the 'vannus' type, rechristening it 'single-vannus' (Fig. 1a), but he subdivided the 'eurystomus' type into three distinct patterns, depending on the position of the interkinetal boundary. In the 'double-eurystomus' type (Fig. 1b), that boundary is centrally situated, while in the two 'double-patella' types (Figs 1c, 1d), it is displaced to either the left or the right, respectively. The classical species *E. eurystomus* possesses the 'double-eurystomus' dargyrome, while *E. patella* possesses the first variant (Fig. 1c) of the 'double-patella' dargyrome. Tuffrau's 'muscicola' dargyrome type was considered by Curds to consist in a heterogeneous mixture of two types: the 'multiple' dargyrome (Fig. 1e), in which there are several rows of polygons between kineties (as in *E. muscicola* Kahl, 1932), and the 'complex' dargyrome (Fig. 1f), in which the interkinetal space is irregularly subdivided (as in *E. elegans* Kahl, 1932).

The ramifications of this typology are not without consequence to the taxonomy of the genus. Dargyrome pattern is one of the few classical taxonomic attributes still considered to be invariant within species of the genus (Carter, 1972; Curds, 1975; Hill & Reilly, 1976) and it remains, for example, one of the major distinguishing features applicable to the confusing assemblage of common freshwater species which includes, in addition to the classical *E. patella* and *E. eurystomus*, others such as *E. aediculatus* Pierson, 1943, *E. plumipes* Stokes, 1884, *E. variabilis* Stokes, 1887 and *E. woodruffi* Gaw, 1939 (Pierson, 1943; Tuffrau, 1960; Pierson *et al.*, 1968; Carter, 1972; Curds, 1975; Hill & Reilly, 1976; Curds, 1977). All of these species are of cirrotype-9

* It should be noted that contrary to the publications of several recent authors, the valid date of publication for the genus *Euplotes* is 1831 (see Ehrenberg, 1831 : 12) not 1830 nor 1838. Furthermore, the 1830 date refers to the nominal publication date of the name *Euploea* for which the correct citation, according to Article 21 of the International Code of Zoological Nomenclature should be *Euploea* Ehrenberg, 1832.

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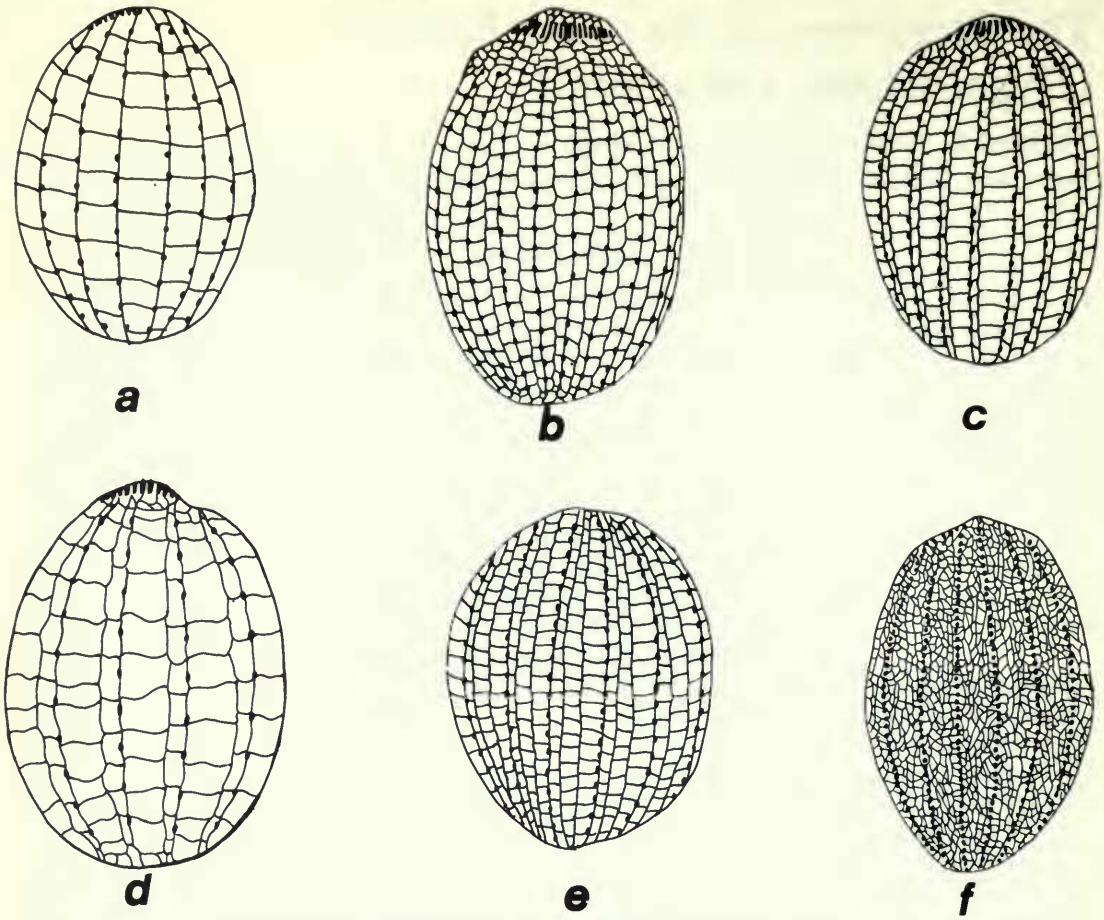

Fig. 1 Dargyrome patterns of *Euplotes*: (a) single-vannus type; (b) double-eurystomus type; (c–d) double-patella types; (e) multiple type; (f) complex type.

(i.e. they have 9 frontoventral cirri), and all of them have very similar cirral patterns on their ventral surface (Gates, 1976).

This paper demonstrates that the subclassification of double dargyromes according to whether the interkinetal boundary is central (the 'double-eurystomus' type) or displaced to the right or left (the 'double-patella' type) is invalid.

Materials and Methods

In March 1975, a clonal population was established in Toronto, Ontario, Canada, of a double dargyrome, cirrotype-10 marine form, labelled QVANNQ (Gates, 1976), which was collected in 1974 from the North Sea off the coast of Denmark and cultured at the British Museum (Natural History). In April 1976, three subclones (1, 2, 3) of this clone were established at the latter institution, and these were sampled at three separate times: A, 11 August 1976; B, 7 December 1976; C, 12 January 1977. These nine samples were silver-stained by a modification of the Chatton–Lwoff procedure (Chatton & Lwoff, 1930; Corliss, 1953; Frankel & Heckmann, 1968).

Within the same microscope slide of a silver-stained preparation of subclonal sample B-1, for example, are found not only typical 'double-eurystomus' specimens (Fig. 2), which are in the majority, but also occasionally a typical 'double-patella' specimen (Fig. 3), plus all of the inter-

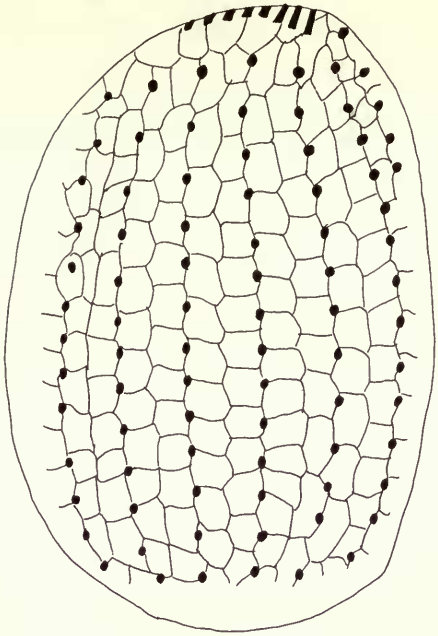

Fig. 2 Dargyrome of a specimen of subclone B-1 of the marine cirrotype-10 *Euplotes* sample, QVANNQ, showing a classical 'double-eurystomus' type of dargyrome.

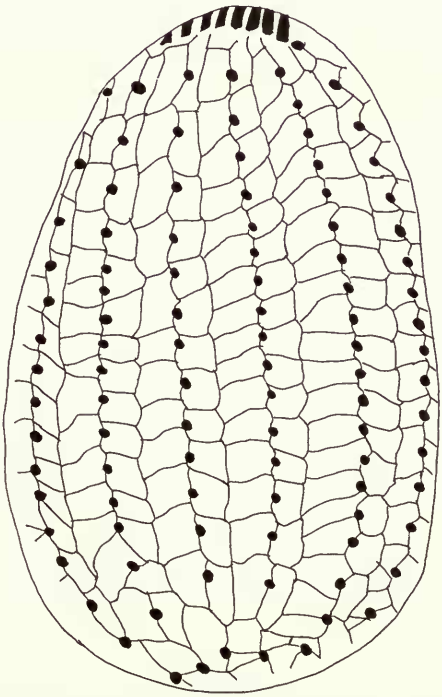

Fig. 3 Dargyrome of another specimen of subclone B-1 from the same slide preparation, showing a classical 'double-patella' type of dargyrome.

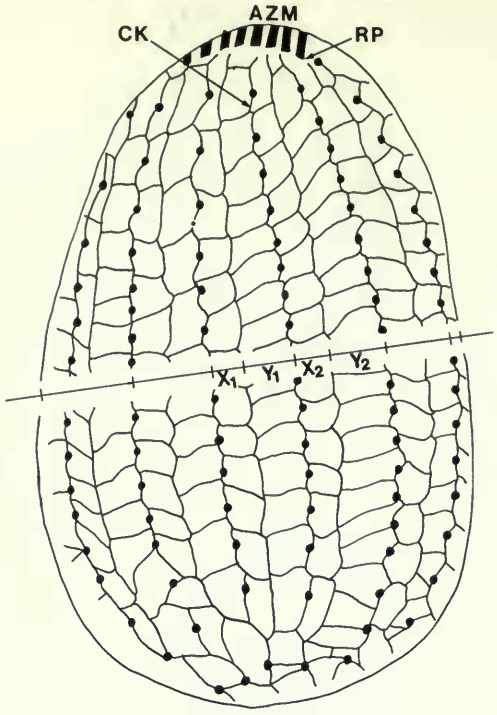

Fig. 4 Diagram of the dorsal surface of a specimen of the QVANNQ *Euplotes* sample, illustrating the measurement transect that provides the two sets of interkinetal distances (x_1, y_1 and x_2, y_2) on either side of the central kinety (ck) that are used to obtain the interkinetal ratios, x_1/y_1 and x_2/y_2, whose average provides a measure of dargyrome type for the specimen. The dorsal origin of the adoral zone of membranelles (AZM) is also shown at the anterior of the specimen, the reference point is indicated by RP.

mediate types. While these examples are suggestive, they are not, in themselves, sufficient to establish the invalidity of a typology. Since evolution is the partitioning of variation, and as variation is quantitative in nature, a detailed quantitative analysis is essential.

As inspection of either Fig. 2 or Fig. 3 reveals, the position of the interkinetal boundary varies with the region of the dorsal surface examined. To obtain an objective, repeatable measure of the position of that boundary within a given population of a double-dargyrome *Euplotes* species, attention must be focused on a restricted portion of the middle of the dorsal surface of well-stained and properly-oriented specimens. The origin of the adoral zone of membranelles (AZM) provides a well-defined reference point (see Fig. 4) on the dorsal surface of this species of *Euplotes*. This point can be used to locate and define the central kinety which is the second kinety lying to the left of the reference point. Accordingly, the widths of the two interkinetal boundaries were measured on either side of the central kinety midway along each specimen.

Using a Leitz Laborlux microscope with 100× oil immersion objective and 10× oculars equipped with a 1·25× drawing tube, the images of the points illustrated in Fig. 4 were recorded on acetate sheets with indian-ink and these were then projected onto millimetre-ruled linear graph paper by means of a Leitz Diascriptor 4 projector. From these coordinate data, the two sets of interkinetal distances (x_1, y_1 and x_2, y_2) determined by the placement of the two central interkinetal boundaries were calculated and the ratios x_1/y_1 and x_2/y_2 formed. Each of these ratios measures the placement of the interkinetal boundary on either side of the central kinety, and their average provides a quantitative measure of the dargyrome of the specimen. Thus, the nature of the dargyrome on each specimen is represented by a single number, the average interkinetal ratio. To measure the dargyrome type of any given population, the mean interkinetal ratio (MIR) is

calculated, based on 50 specimens for each sample, except series A-1, where only 19 specimens were available.

Comparisons among populations were made by means of standard analysis of variance tests, primarily the *F*-ratio test (Sokal & Rohlf, 1969). A 95% confidence level was used throughout. Conclusions were not affected by assuming that the average interkinetal ratio is normally distributed within each population (as was verified to be true in one sample): use of the arcsine transformation for ratios (Sokal & Rohlf, 1969) gave similar results to those presented here, which are based on untransformed data. To facilitate comparisons, results are given in terms of the 95% confidence intervals (on either side of the mean) for each sample, based on *t*-values, rather than the *F*-ratios for each group of samples.

Results

An essential ingredient in any quantitative analysis of variation is an examination of the inherent errors of measurement. Table 1 presents the results of measuring the same specimen 50 times, which gives a measurement error confidence interval for the MIR in the order of 0·015. Also shown are the results of measuring 50 specimens of each of two separate slide preparations of the B-1 sample; it is clear that there are no differences among subsamples of this sample. These results demonstrate, respectively, the precision and accuracy of the MIR as a measure of dargyrome type.

Table 1 Error analysis for the mean interkinetal ratio. Using the B-1 subclone, the same specimen was remeasured 50 times to assess the inherent error of measurement. To judge the accuracy of measurement, 50 specimens on each of two separate slides of this same subclone were measured. In this and the two following tables, the mean ($\bar{x}$), standard deviation (s) and 95% confidence interval (*c.i.*) are presented

	$\bar{x}$	s	*c.i.*
50 replicates	1·0546	0·0505	0·0144
Slide 1	1·0099	0·0972	0·0276
Slide 2	0·9906	0·0990	0·0281

As Figs 2 and 3 clearly illustrate, there can be considerable variation from individual to individual in dargyrome type. But to demonstrate the invalidity of the concept, it must be established that populations of individuals differ, not merely that individuals vary. Given the mean interkinetal ratio of a population, it must be established, first, that the ratio is inherited, and second, that it is sufficiently variable that different subclones may have significantly different ratios. That is, if two individuals are derived from a parental clonal population, are their ratios different, and will those ratios be inherited by their asexual progeny ?

Tables 2 and 3 present the results. For each clone, there are no significant differences in the MIR with time, over the period sampled (Table 2). This demonstrates that the MIR is inherited within clones. If the results are regrouped by fixation date (Table 3), it is apparent that significant differences do exist among the three clones. Clone 1 is always larger in MIR than clone 3, and clone 2 is always intermediate; the differences between clone 1 and clone 3 are significant at the last two fixation times, B and C. This consistent and significant trend shows that different subclones may possess significantly different ratios.

Discussion

The results demonstrate that, although the asexual progeny of an individual inherits the dargyrome type of the parent, different individuals may give rise to progeny which have significantly

Table 2 Mean interkinetal ratios for the 9 subclones, arranged by clone. For each clone, there are no significant differences among the three different sampling times (A, B, C)

Clone	$\bar{x}$	s	*c.i.*
A-1	0·9790	0·1657	0·0471
B-1	1·0099	0·0972	0·0276
C-1	1·0760	0·1629	0·0463
A-2	0·9485	0·1525	0·0433
B-2	0·9527	0·1074	0·0305
C-2	0·9977	0·1837	0·0522
A-3	0·9194	0·1471	0·0418
B-3	0·9072	0·1189	0·0338
C-3	0·9477	0·1695	0·0482

Table 3 Mean interkinetal ratios for the 9 subclones, arranged by sampling time. Significantly different clones within each sampling time are indicated by asterisks on the means

Clone	$\bar{x}$	*c.i.*
A-1	0·9790	0·0471
A-2	0·9485	0·0433
A-3	0·9194	0·0418
B-1	1·0099*	0·0276
B-2	0·9527	0·0305
B-3	0·9072*	0·0338
C-1	1·0760*	0·0463
C-2	0·9977	0·0522
C-3	0·9477*	0·0482

different dargyrome types, as measured by the MIR. This form of quantitative uniparental inheritance of the mean interkinetal ratio makes its use as a taxonomic criterion invalid. More poignantly, qualitative distinctions based on the same principle are likewise invalid: the 'double-eurystomus' and 'double-patella' dargyrome types do not exist as separate entities. They represent extremes of a continuum of interkinetal ratios that exists within most large populations of double dargyrome *Euplotes*. The typological fixation of the extremes of this continuum is to be deplored.

The work of Frankel (1973, 1975) demonstrates the considerable independence of the ventral and dorsal surfaces of *Euplotes*. In another marine cirrotype-10 species, *E. minuta* Yocom, 1930, the distribution of ciliary units over the dorsal surface is stable, but there can be large variations both in the number of kineties and in the number of cilia per kinety, while on the ventral surface, the number of frontoventral cirri (the cirrotype) remains constant, even in a basal-body deficient mutant. The present study is also based on a marine cirrotype-10 form, and the most parsimonious interpretation of our results is that other double dargyrome *Euplotes* do have a similar quantitative uniparental inheritance of the MIR. In particular, we suggest that it applies equally well to the cirrotype-9 freshwater forms such as *E. patella* and *E. eurystomus*, which display similar inidvidual variations in MIR (Gates, unpublished observations). In conjunction with the results of quantitative studies of the ventral cirral patterns of these forms (Gates, 1976), such an interpretation is of obvious applicability to the alleviation of the taxonomic confusion which exists among freshwater cirrotype-9 *Euplotes* (see, for example, Curds, 1975; Hill & Reilly, 1976).

The existence of individual variation in dargyrome type is not confined to samples of *Euplotes* having double dargyromes (Gates, 1976, and unpublished observations). Among individuals of

various single dargyrome forms, one may see not only the erratic 'reorganizing' dargyromes which Tuffrau (1960) noted in his *E. mutabilis* Tuffrau, 1960, now known to be fully interfertile with *E. crassus* (Dujardin, 1841) Kahl, 1932 (Génermont *et al.*, 1976), but also occasional regions in which a double dargyrome pattern is present, or in which the interkinetal space is further subdivided. The latter phenomenon also occurs in various double dargyrome samples, including *E. harpa* Stein, 1859. Indeed, among some double dargyrome forms, multiple or complex patterns occur in restricted regions of the dorsal surface of a few individuals, while in *E. moebiusi* Kahl, 1932 (Curds, 1974) and *E. tegulatus* Tuffrau, 1960 (Tuffrau, 1960) there is a more general complication of the dargyrome in all specimens examined. Finally, some 'multiple' dargyrome clonal samples yield a few specimens having classical 'complex' dargyromes, suggesting that the latter type is only a variant of the former.

While all of these variants and exceptions are rare, they are as suggestive as Figs 2 and 3. Indeed, they suggest that dargyrome types are not immutable, and that it is possible to suggest an evolutionary sequence from 'single' through 'double' to 'multiple' (with its variant, 'complex') dargyromes (Gates, 1976). That is, the direction of evolutionary change has been the further subdivision of the interkinetal space by the corresponding polymerization of subpellicular vacuoles (see Polyjansky & Raikov, 1976).

The 'single' dargyrome occurs only in small to medium-sized marine species of cirrotype-10, and these forms have identical cirral patterns (Gates, 1976). The 'double' dargyrome is the most common type, found in both marine and freshwater forms of all cirrotypes; it occurs among species with a variety of cirral patterns and sizes; and it is the most variable dargyrome. The 'multiple' and 'complex' dargyromes are restricted to only a few species (Curds, 1975).

The common occurrence and the variable nature of the 'double' dargyrome has led to the creation of subcategories which were presumed by taxonomists to be stable within 'species' (Curds, 1975). Our results demonstrate the invalidity of the subdivision using this type of dargyrome. Although descriptive of particular clones, the 'double-patella', 'double-eurystomus' and 'complex' distinctions, should not be used in assessing taxonomic affinities. Because of the quantitative uniparental inheritance of dargyrome type, these categories are not descriptive of populations of *Euplotes*, even over short periods of time. Accordingly, we propose to modify Tuffrau's (1960) original classification along the more descriptive lines suggested by Curds (1975). The dargyrome of *Euplotes* should be classified as either 'single', 'double' or 'multiple'.

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